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Association of Serum Lipoprotein(a) With Ultrasonographically Determined Early Atherosclerotic Changes in the Carotid and Femoral Arteries in Kidney Transplanted Patients

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ABSTRACT

Objectives. To evaluate the association of serum lipoprotein(a) [Lp(a)] with carotid intimal media thickness (IMT) and carotid femoral plaque occurrence in kidney transplant patients.

Patients and Methods. Fifty-four subjects included 29 group 1 normal healthy persons and 25 group 2 kidney transplant patients underwent carotid IMT measurements and carotid femoral plaque assessment by B-mode ultrasonography. Also we measured cholesterol, triglyceride, HDL-C, LDL-C and Lp(a) as well as BUN and creatinine.

Results. There was a significant difference between Lp(a) in the two groups ($P = .016$). There was a significant difference between carotid IMT of the two groups ($P < .001$). Moreover there was a significant difference between the plaque scores of kidney transplant patients and the normal group ($P = .05$). There were no correlations between carotid IMT and plaque score in normal subjects or in kidney transplant patients ($P > .05$). There was a significant correlation between carotid IMT with age in Group 1 ($P = .035$). No correlation between carotid IMT and serum Lp(a) was seen in the two groups. No significant correlations between plaque score and serum Lp(a) were observed. There was no correlation between duration of transplant and thickening of intimal media complex in this group. In this group a positive correlation was demonstrated between carotid IMT with serum LDL-C ($P < .001$).

Conclusions. Age was the most important factor associated with thickening of intimal media complex in normal subjects and in plaque formation in the renal transplant group. Serum LDL-C may be associated with thickening of intimal media complex in kidney transplant patients. Serum Lp(a) may not be a significant factor in thickening of the intimal media complex or plaque occurrence in kidney transplant patients.

COMPARED with the general population, kidney transplant patients suffer from excessive mortality due to atherosclerotic cardiovascular disease (ACVD). This observation is frequently attributed to a process of accelerated (rapidly progressive) atherosclerosis; since the risk factors for ACVD, such as hypertension, lipid abnormalities, glucose intolerance, and left ventricular hypertrophy, are more commonly observed in this patient group.[1,2] Lipoprotein(a) [Lp(a)] when present in high levels in plasma, has been recognized as an independent risk factor for premature atherosclerotic coronary heart disease.[3] Lp(a), a cholesterol-rich particle in human plasma, was first described by Berg in 1963.[2–4] Many epidemiologic and case-control studies have shown high levels of plasma Lp(a) to be an independent risk factor for premature atherosclerotic coronary heart disease.[2–4] The exact mechanism by which

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Lp(a) is a cardiovascular risk, is unknown. Although both proatherogenic and prothrombogenic effects have been hypothesized, the biological role and normal metabolism of this lipoprotein have not been fully elucidated.[2–4] Increased Lp(a) levels may be a contributing factor to an increased incidence of atherosclerotic disease in kidney transplant patients.

The early stages of atherosclerosis are associated with subtle structural changes in arteries, such as thickening of arterial intimal media complex (IMT).[5–7] Carotid IMT is increased among subjects with several risk factors predicting cardiovascular events and target organ damage.[8] B-mode ultrasonography to assess early atherosclerosis is a safe and noninvasive method to study superficial vascular structures, such as the carotid and femoral arteries.[6–8] Therefore, ultrasound evaluation of the carotid artery for IMT as well as for plaques may identify patients at risk for cardiovascular disease.[7,8] Indeed carotid arteries are a privileged area to study the progression of atherosclerotic lesions from onset to fully developed plaque. Carotid IMT measurements are strongly related to the extent of atherosclerosis in other vascular districts too.[7,8] Conventional risk factors have been shown to be significantly associated with increased arterial wall thickness, consistent with their accepted role in atherogenesis; much less is known, however, about the effects of serum Lp(a) on IMT in renal transplant patients. We therefore sought to study the effects of serum Lp(a) levels on early structural atherosclerotic vascular changes in a group of kidney transplant patients. The second aim was to correlate carotid artery IMT in the two groups with other certain risk factors.

SUBJECTS AND METHODS

This cross-sectional study was performed on healthy control subjects (Group 1) and kidney transplant patients (Group 2). Subjects were evaluated with lipid measurements and B-mode ultrasonographic evaluation of carotid arteries for IMT and carotid femoral arteries for plaque occurrence. Exclusion criteria included cigarette smoking, body mass index (BMI) above 25, hypolipemic drugs, diabetes mellitus, recent MI, vascular disease, and active or chronic infection. Group 1 subjects had no history of hypertension or renal disease. Group 2 were renal transplant patients under cyclosporin, prednisolone, and mycophenolate mopheti (Cellcept) or Imuran therapy. For laboratory evaluation, subjects fasted overnight for 14 hours. Fasting blood sugar (FBS), Lp(a), triglyceride (TG), cholesterol (Chol), HDL-C, LDL-C, BUN, and creatinine were measured. Serum Lp(a) was estimated by enzyme-linked immunosorbent assay (ELISA); Immuno-Biological Laboratories [IBL]. Kit (Hamburg, Germany). Other lipids, BUN, creatinine, and FBS were measured by standard kits. Serum LDL-C was calculated by Friedewald's formula[9]; and creatinine clearance by serum creatinine, age and body weight.[10] Prior to consent Group 1 subjects were interviewed using a questionnaire to ascertain that they were free of clinical evidence or history of diabetes, cardiac or vascular disease, and had no past or current history of hypertension. The clinical history of patients was determined from hospital medical records. All kidney transplant patients who were hypertensive were near control or completely controlled by antihypertensive therapy. Carotid and femoral artery ultrasonography were performed by a single technician unaware of the history or laboratory data of the patient. IMT was measured using a Honda-Hs-2000 Sonograph with 7.5-MHz linear probe. The procedure was performed at the end of the diastolic phase. The sites of measurements were at the distal common carotid artery, the area of bifurcation and at the first proximal internal carotid artery. IMT was measured in plaque-free areas with the subjects in the supine position with neck hyperextension and rotation of the head to facilitate the procedure. The carotid was evaluated in the axial longitude. By sonography, the carotid artery was found to have three different echoes. Intimal is the echogenic layer line; media the hypoechoic; and adventitia, the echogenous. IMT was defined as the distance from leading edge of the lumen–intimal interface of the far wall to the leading edge of the media adventitiae interface of the far wall. IMT more than 0.8 mm was considered abnormal. For statistical analysis we measured the mean of the right and the left carotid IMT. Sonography for plaque, performed on the right and left carotid and femoral arteries, was scored from 0 (no plaque) to 4 (plaque present at all four sites), regardless of the number and size of the plaques at each site, plaque occurrences in each site being scored with 1 point. Plaques were divided into three groups: soft, calcified, or mixed. Plaques were visualized as echogenic or hypoechoic protrusions into the vessel lumen. Plaques was considered to be local intimal thicknesses more than 1 mm. For plaque measurements the largest longitude was considered.

For statistical analysis descriptive data were expressed as Mean values ± SD and frequency distributions. Comparison between groups were performed using T and chi-square tests. For comparisons between plaque scores in the two groups, a chi-square test was used. For correlations we used regression tests with a stepwise method. Statistical significance was inferred at p values < 0.05. All statistical analyses were performed using the statistical analysis system (SPSS version 11.00).

RESULTS

The 54 patients consisted of 29 (15 female and 14 male) healthy subjects and 25, (16 female and 9 male) renal transplant patients were evaluated. Table 1 shows the frequency distribution of age (year); duration of disease, (DD) namely, the length of time the patients had received a transplanted kidney in months; and creatinine clearance (CLcr). CLcr among Group 1 was 103 ± 5.5 mL/min and among kidney transplant patients was 50.0 ± 8.6 mL/min. Table 2 shows the frequency distribution of carotid–femoral plaque scores. Table 3 shows the frequency distribution of lipids and carotid IMT. Mean ± SD of Lp(a) in Groups 1 and 2 were 42 ± 20 mg/dL and 54 ± 20 mg/dL, respectively. Carotid IMT in Groups 1 and 2 were 0.88 ± 0.18 mm and

Table 1. Frequency Distribution of Age, Duration of Disease and Creatinine Clearance

Variables		Mean ± SD	Minimum	Maximum
Group 1	Age (y)	45.0 ± 10.4	20	70
	DD (mo)	—	—	—
	CLcr (cc/min)	103 ± 5.5	100	120
Group 2	Age (y)	42.8 ± 12.4	20	70
	DD (mo)	63.26 ± 36.00	4	132
	CLcr (cc/min)	50.0 ± 8.6	30	65

Abbreviations: DD, duration of disease; CLcr, creatinine clearance.

Table 2. Frequency Distribution of Plaque Scores

Plaque Score	Frequency	Percent
Group 1		
0	27	93.0
1	1	3.4
2	1	3.4
3	0	0.0
4	0	0.0
Group 2		
0	17	68.0
1	6	24.0
2	2	8.0
3	0	0.0
4	0	0.0

1.10 ± 0.22 mm, respectively. Ultrasound assessments for plaque score were: 6.80% of Group 1 with plaques of scores 1 to 2; 32% of Group 2 had plaque in scores of 1 to 2. There was no plaque occurrence of scores 3 or 4 in either group 5. Although there was a significant difference in the serum Lp(a) unless between the two groups ($P = .032$). There were significant differences in TG ($P = .003$), and cholesterol ($P = .036$) between the two groups. There were no significant differences in age ($P = .436$), HDL-C ($P = .505$), and LDL-C ($P = .446$) between the two groups (*t*-test). There was a significant difference in carotid IMT between normal subjects and the kidney transplant group ($P < .001$; *t*-test). A significant difference in plaque score between Group 1 and Group 2 was observed ($P = .050$; Chi-square test). By ANOVA, there were no correlations between carotid IMT with plaque score in either normal subjects or kidney transplant patients ($P > .05$). There was a significant correlation between carotid IMT with age in Group 1 ($P = .035$), but not in Group 2 ($P > .05$). By the regression method there was no significant correlation between constant variables (LDL-C, HDL-C, Lp(a), Tg, Chol, gender) and carotid IMT (dependent variable) in Group 1. Among Group 2, there was a positive correlation between carotid IMT with LDL-C ($P < .001$; stepwise regression analysis). No significant correlation between carotid IMT and other variables consisting of lipids [HDL-C, Chol, Triglyceride and serum L(a)], duration of disease or CLcr was demonstrated in the renal transplant group. No significant correlation was found between constant variables of Chol, HDL-C, LDL-C, Tg, Lp(a), age, or gender and plaque score in Group 1 or 2 ($P > .05$). A significant correlation between plaque score and age was found in Group 2 ($P = .001$).

Table 3. Frequency Distribution of Lipids (mg/dL) and IMT (mm)

Variables	Mean ± SD	Minimum	Maximum
Group 1 ($n = 29$)			
Lp(a)	42.0 ± 20.0	10	94
Chol	203 ± 41	125	340
LDL-c	126 ± 34	75	230
HDL-C	41 ± 10	25	65
Tg	154 ± 73	50	325
IMT	0.88 ± 0.18	0.5	1.20
Group 2 ($n = 25$)			
Lp(a)	54.0 ± 20.0	14	90
Chol	231 ± 52	150	360
LDL-C	134 ± 41	80	260
HDL-C	39 ± 11	20	65
Tg	235 ± 112	85	520
IMT	1.10 ± 0.22	0.90	1.90

Abbreviations: Chol, cholesterol; HDL-c, high-density lipoprotein cholesterol; IMT, intimal media thickness; LDL-c, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); Tg, triglycerides.

DISCUSSION

The principal findings of this study were higher levels of Lp(a), carotid IMT, and arterial plaques in kidney transplant patients compared with normal subjects. No association of carotid IMT with carotid-femoral plaque score was seen. A positive correlation of carotid IMT with age was seen in normal subjects but not in kidney transplant patients. No significant correlation was found between carotid IMT with serum Lp(a) in the two groups. Moreover, no significant correlation was observed between plaque score and serum Lp(a) in the two groups. There was no correlation between duration of time of receiving a transplanted kidney and thickening of the intima-media in this group.

Studies concerning the effect of Lp(a) on thickening of media/intimal complex and plaque occurrence in normal subjects and in patients who have received kidney transplants have shown various results. Sramek et al[11] found no increased IMT in the carotid or femoral artery at high levels of Lp(a) among 142 asymptomatic men. He concluded that serum Lp(a) levels are not associated with early atherosclerotic vessel wall changes in the carotid or femoral arteries.[11] In a study on 100 elderly subjects (aged 78.5 ± 0.6), Dentil et al showed no association between carotid IMT and Lp(a), concluding that Lp(a) was unrelated to the severity of extracranial vessel atherosclerosis.[12] Baldassarre et al, in a study on 100 type 2 hypercholestrolemic patients, observed higher values of carotid IMT in hyercholesterolemic patients and serum Lp(a) levels >30 mg/dL than among those with lower levels. He concluded that elevated plasma levels of Lp(a) may be considered an additional independent factor associated with thickening of the carotid artery among patients with severe hypercholesterolemia, but not those with moderate hypercholesterolemia or normocholeseolemic subjects.[13] Among 241 healthy subjects Raitakari et al suggested no association between IMT and Lp(a), but significant positive correlation with total cholesterol, LDL-C, LDL/HDL ratio, age, and TG.[4] Moreover, a positive correlation of IMT with age in 10,002 normal subjects was shown by Dobs et al.[14] Suwelack et al showed greater thickening of IMT in a group of kidney transplant patients (35 patients), he could not show any association between age or Lp(a) with IMT in his study.[1] Among 19 kidney transplant recipients Jogestrand showed more plaque occurrence in the carotid arteries of patients with

hyperlipidemia.[15] Barbagallo et al in a study on 57 renal transplant recipients demonstrated an increased incidence of plaque compared to normal controls; the lesions were independent of age and also showed no correlation with plasma lipoproteins and carotid lesions.[16] In the present study, we did not observe an association between carotid IMT or carotid femoral plaques with serum Lp(a), although this group showed higher serum values of Lp(a), increased thickening of intima–media complex and more plaque occurrence. It seems that Lp(a) may not be a significant factor in thickening of the intima–media complex and plaque occurrence in kidney transplant patients. In the meantime further clinical study into this important aspect of kidney transplanted patients is needed.

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